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Resistance to Fever Induction and Impaired Acute-Phase Response in Interleukin-1β-Deficient Mice

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Summary

We used gene targeting in embryonic stem cells to introduce an IL-1β null allele in mice. The IL-1β-deficient mice develop normally and are apparently healthy and fertile. The IL-1β null mice responded normally in models of contact and delayed-type hypersensitivity or following bacterial endotoxin LPS-induced inflammation. The IL-1β-deficient mice showed equivalent resistance to Listeria monocytogenes compared with wild-type controls. In contrast, when challenged with turpentine, which causes localized inflammation and tissue injury, the IL-1β mutant mice exhibited an impaired acute-phase inflammatory response and were completely resistant to fever development and anorexia. These results highlight a central role for IL-1β as a pyrogen and a mediator of the acute-phase response in a subset of inflammatory disease models, and support the notion that blocking the action of a single key cytokine can alter the course of specific immune and inflammatory responses.

Introduction

Mammalian interleukin-1 (IL-1) is a proinflammatory cytokine that is produced particularly by mononuclear phagocytes, but also by numerous other cell types, in response to injury and infection (reviewed by di Giovine and Duff, 1990; Dinarello, 1992; and references therein). The biological activity of IL-1 is encoded by two distinct genes, termed IL-1α and IL-1β, which share approximately 25% primary amino acid sequence identity. These two gene products, however, have similar three-dimensional structures, bind to the same receptors on target cells, and elicit identical biological responses (Sims et al., 1988; Chizzonite et al., 1989). IL-1β is believed to be the major form of IL-1 released from cells, while IL-1α is thought to be primarily cell associated (Denczuk et al., 1987; Brody and Durum, 1989). IL-1α and IL-1β are both synthesized as 31.5 kDa precursor proteins, and are proteolytically cleaved to generate mature proteins of 17 kDa (Cameron et al., 1986; Kostura et al., 1989). Both pro-IL-1α and mature IL-1α are active, whereas precursor IL-1β is biologically inactive (Brody and Durum, 1989). The maturation of precursor IL-1β to its mature form involves an endoproteinase termed IL-1β-converting enzyme (ICE) (Thornberry et al., 1992; Cerretti et al., 1992).

IL-1 has been implicated in a broad spectrum of inflammatory, physiologic, hematopoietic, and immunologic activities. It is believed to be a mediator of inflammation in both man and animal models and has been proposed as an attractive target for therapeutic intervention in the treatment of inflammatory diseases. IL-1 is produced in response to systemic and local inflammation and contributes to loss of body weight, anorexia, and hepatic acute-phase protein synthesis (Beisel, 1975; Kushner and Mackiewicz, 1993). IL-1 has been shown to be an important endogenous pyrogen, since administration of recombinant IL-1 results in an increase in body temperature and neutralizing antibodies against IL-1β partially inhibits lipopolysaccharide (LPS)-induced fever (Kluger, 1991; Long et al., 1990). Studies using neutralizing antibodies have shown that IL-1, in particular IL-1β, is involved in the development and progression of collagen-induced arthritis (CIA) in mice (Geiger et al., 1993; Van den Berg et al., 1994). IL-1β is rapidly accumulated in Langerhans cells after exposure to contact allergens and is believed to play a critical role in the initiation of contact hypersensitivity (CH) responses in the skin (Enk and Katz, 1991; Enk et al., 1993). In contrast with its role in inflammatory diseases, IL-1 has been implicated as a mediator in host defense (Van der Meer et al., 1988; Havell et al., 1992). IL-1 has been shown to participate in the development of anti-Listeria responses in mice and administration of IL-1 prior to or during bacterial infection enhanced antimicrobial activity and survival (Van der Meer et al., 1988; Rogers et al., 1992).

The expression of IL-1 is under stringent control. IL-1 activity is usually undetectable in normal biological fluids, but can be transiently induced in response to infection, inflammatory stimuli, and tissue injury. The activity of IL-1 is regulated at the transcriptional, posttranscriptional, and posttranslational levels (reviewed by Fenton, 1992 and references therein). IL-1 interacts with cells through two classes of molecules: type I and type II receptors (Dower and Urdal, 1987). Although both IL-1α and IL-1β bind the two receptors with similar affinities, the type II receptor

may function as a decoy target for IL-1 and inhibit its biological activity (Colotta et al., 1993). In addition, a unique naturally occurring IL-1 receptor antagonist (IL-1ra) is expressed by many cell types and functions as an IL-1 inhibitor by binding the IL-1 receptor without triggering any biological response (Eisenberg et al., 1990).

Although IL-1 is regarded mainly as an inducible gene, it is constitutively expressed in several tissues, such as skin keratinocytes and cells of central nervous system (CNS) origin. IL-1β activity can be detected in the hypothalamus and may act as a neurotransmitter (Breder et al., 1988). In addition, IL-1 is expressed during the preimplantation period, suggesting that it may play a role during early embryogenesis (McMaster et al., 1992; Rothstein et al., 1992).

Despite extensive studies in vitro, the physiological importance of IL-1 in vivo remains largely unknown. The specific function of IL-1 is further obscured by the fact that many of its activities overlap with those of other cytokines, primarily tumor necrosis factor-α (TNFα) and IL-6. These cytokines regulate one another to form a cytokine network (Akira et al., 1990). For example, IL-1 and TNFα are capable of inducing each other and are both potent inducers of IL-6. To address unequivocally the in vivo function of IL-1β, we produced an IL-1β-deficient mouse strain by homologous recombination. We show here that the IL-1β null mice were unable to mount a normal acute-phase inflammatory response following turpentine injection, which causes localized leukocyte infiltration, edema and tissue damage. The loss of body weight and elevation of IL-6 level typically associated with this model were not observed in the mutant mice. Most dramatically, the IL-1β-deficient mice did not develop fever following turpentine administration. In contrast, the IL-1β-deficient mice responded similarly to wild-type controls in systemic inflammation induced by LPS injection, infection with L. monocytogenes, and in models of CH and delayed-type hypersensitivity (DTH).

Results

Generation of IL-1β-Deficient Mice

To generate a null allele of the IL-1β gene in mouse embryonic stem (ES) cells, we constructed a targeting vector in which the first 6 of 7 exons of the IL-1β gene were deleted and replaced with the PGK-*neo* sequence (Figure 1A). The 5′ and 3′ homologies were 4.5 kb and 1.3 kb, respectively. The HSV-*tk* cassette was attached at the end of the 3′ homology for negative selection (Mansour et al., 1988). The linearized vector was electroporated into AB2.1 mouse ES cells (Matzuk et al., 1992) and the transfected cells were selected in G418 and FIAU. An 8-fold enrichment was achieved by FIAU counterselection as compared with G418 selection alone. G418- and FIAU-resistant colonies were expanded and analyzed by a mini-Southern protocol (Ramírez-Solis et al., 1992). A total of three targeted clones were identified from 350 double-resistant clones analyzed. Therefore, the frequency of targeted recombination versus random integration at the IL-1β locus is 1/930. The homologous recombination events were identified by digestion of genomic DNA with EcoRI and KpnI, followed by hybridization with an IL-1β probe (Figure 1A) in a Southern blot analysis. The wild-type IL-1β allele displays a 7.6 kb EcoRI fragment and a 10 kb KpnI band. Correctly targeted recombination events would generate a 3.8 kb EcoRI fragment due to the deletion of the IL-1β gene and a 16 kb KpnI band, since the KpnI site inside the IL-1β gene was mutated in the vector (Figure 1B). Homologous recombination was also determined by hybridization with the *neo* sequence, which confirmed the occurrence of only a single integration event in these clones (data not shown).

The targeted clones were injected into 3.5 dpc C57BL/6J blastocysts to generate chimeras. A total of 9 male and 3 female chimeras were produced with ES cell contributions ranging from 60%–100%, as judged by the percentage of agouti color in the chimeric mice. Male chimeras were bred to C57BL/6J females to assess their potential for germline colonization. Clone 214 and 318 (data not shown) resulted in successful transmission of ES cells into the next generation, of which approximately 50% of the agouti pups contained the deleted IL-1β gene (data not shown). Mice generated from clone 214 were used as the focus for further study. Heterozygous mice were phenotypically indistinguishable from their normal littermates.

To produce mice homozygous for the disrupted IL-1β allele, cross matings between heterozygous mice were set up. Normal litter sizes were observed. The resulting pups were genotyped by Southern blot analysis (Figure 1C). Of 102 mice analyzed at 2 weeks of age, 23 (22.5%) were wild-type (+/+), 54 (53%) were heterozygous (+/−), and 25 (24.5%) were homozygous (−/−) for the deleted IL-1β gene, resulting in a normal 1:2:1 Mendelian distribution of offspring. The ability to generate viable homozygous IL-1β knockout mice ruled out a nonredundant function for IL-1β during mouse embryogenesis. The homozygous IL-1β mutant mice were healthy and fertile in a specific pathogen-free environment. Fluorescence-activated cell sorter (FACS) analysis showed that the T and B cell lineages were not significantly affected in the IL-1β knockout mice. Detailed histopathological evaluation failed to reveal any abnormalities in the mutant mice (data not shown).

LPS-Induced Expression of IL-1β and Other Cytokines

To determine whether a complete deficiency of IL-1β was produced in the mutant mice, we induced IL-1β expression by sensitizing the animals with 1 mg of heat-killed Propionibacterium acnes and by challenging them with an intraperitoneal injection of 10 μg of bacterial endotoxin LPS 6 days later. The mice were sacrificed 3 hr after challenge and blood and peritoneal lavage samples were obtained. Livers were taken for isolation of RNA, and Northern blot analysis was carried out by hybridizing the RNA samples with IL-1β cDNA (Figure 2).

ELISAs were performed on plasma and peritoneal lavage samples to determine the levels of IL-1β as well as IL-1α, IL-6, and TNFα. IL-1β protein could not be identified, although the levels of IL-1α, IL-6, and TNFα were elevated

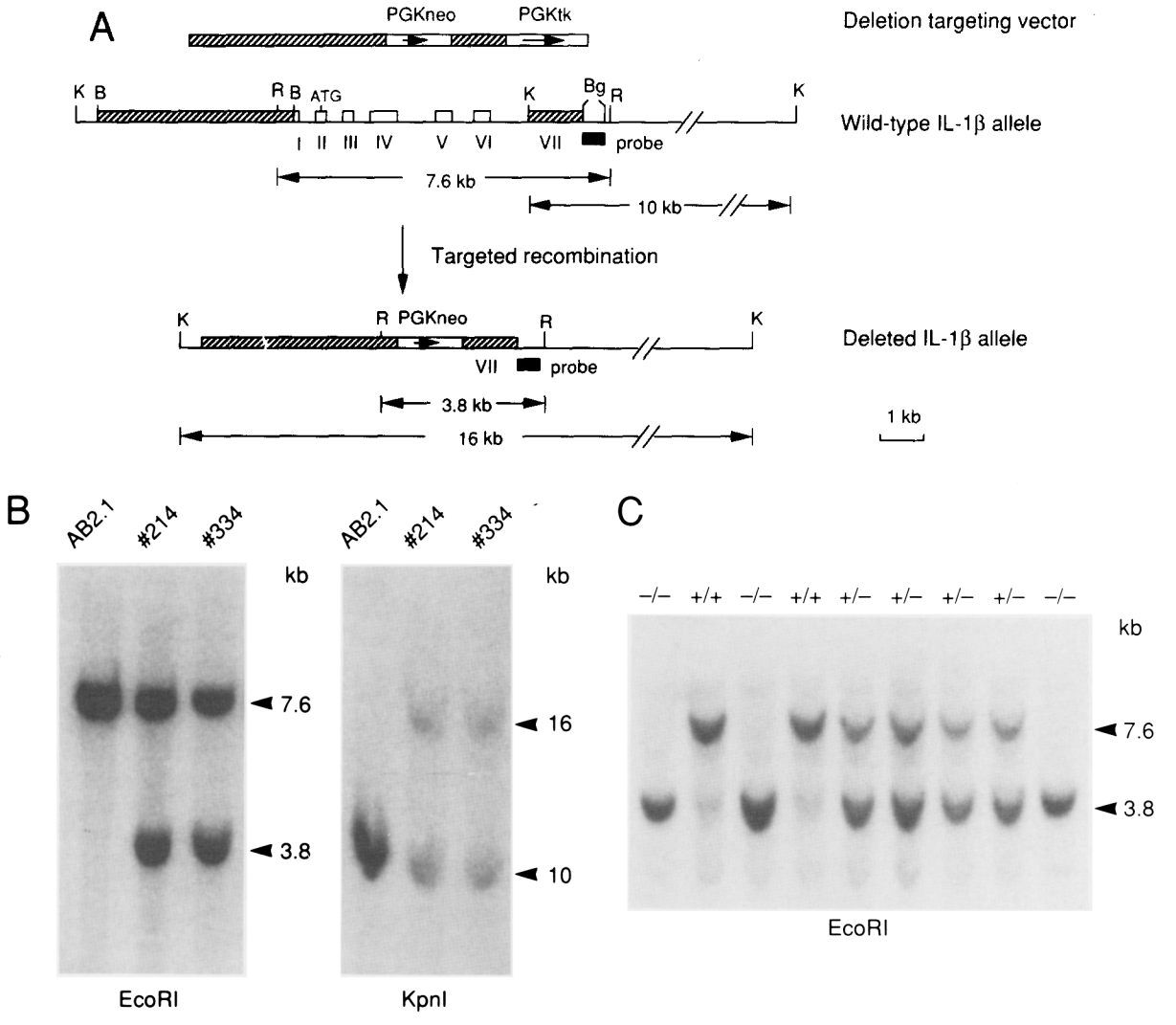

Figure 1. Generation of IL-1β-Deficient Mice by Gene Targeting in Mouse ES Cells

(A) Targeted deletion of IL-1β gene in ES cells. The deletion targeting vector contained a 4.2 kb BamHI fragment upstream of the ATG translation initiation codon and a 1.3 kb KpnI–BglII fragment containing exon 7 of the IL-1β gene as homologous targeting sequences (represented by shaded rectangles). A 5.6 kb BamHI–KpnI fragment from exon 1 to intron 6 of the IL-1β gene was deleted and replaced with the positive selective marker PGK-*neo* (Rudnicki et al., 1992), which was inserted in the same orientation as the IL-1β gene. The PGK-*tk* cassette was attached at the end of the short arm for negative selection with FIAU. Targeted recombination between the vector and the wild-type IL-1β locus results in the deletion of most of the IL-1β coding sequence, followed by its replacement with the *neo* gene. The probe used for detecting the targeted event was a 0.5 kb BglII fragment immediately downstream of the short arm. Digestion with the restriction endonucleases EcoRI and KpnI were used to separate the wild-type and the targeted IL-1β alleles. EcoRI digestion generates a 7.6 kb wild-type band and a 3.8 kb targeted band and KpnI digestion detects a 10 kb wild-type allele and a 16 kb targeted allele. B, BamHI; R, EcoRI; Bg, BglII; K, KpnI. PGK, phosphoglycerate kinase promoter.

(B) Southern blot analysis of targeted clones. ES cell DNA (8 μg) from wild-type AB2.1 cells and two positive clones (214 and 334) were digested with restriction enzyme EcoRI (left) and KpnI (right), electrophoresed on 0.8% and 0.6% agarose gels, respectively, transferred onto Gene Screen Plus nylon membranes (New England Nuclear Dupont), and hybridized with the 0.5 kb BglII probe (see [A]). The diagnostic 3.8 kb EcoRI and 16 kb KpnI bands were detected in both clones.

(C) Southern blot analysis of offspring from heterozygous matings. Genomic DNA isolated from tails of 2-week-old pups generated from crosses of heterozygous mice was digested with EcoRI, transferred to membranes, and hybridized with the IL-1β probe. +/+, wild-type; +/−, heterozygous; −/−, homozygous IL-1β mutant mice.

in response to LPS injection in the IL-1β homozygous mutant mice to a similar extent as in the wild-type controls (Table 1). The creation of a complete null allele was corroborated by demonstrating that IL-1β mRNA, although significantly expressed in response to LPS in the wild-type mice, could not be detected in the knockout animals (Figure 2). The expression of several liver acute-phase proteins, including serum amyloid A (SAA), haptoglobin (HP), α-1 acid glycoprotein (α-AGP), and α-2 macroglobulin (α_2m) were determined by quantitating the RNA levels following LPS induction. Significant differences could not be detected in the amount of mRNA induced for any of the acute-

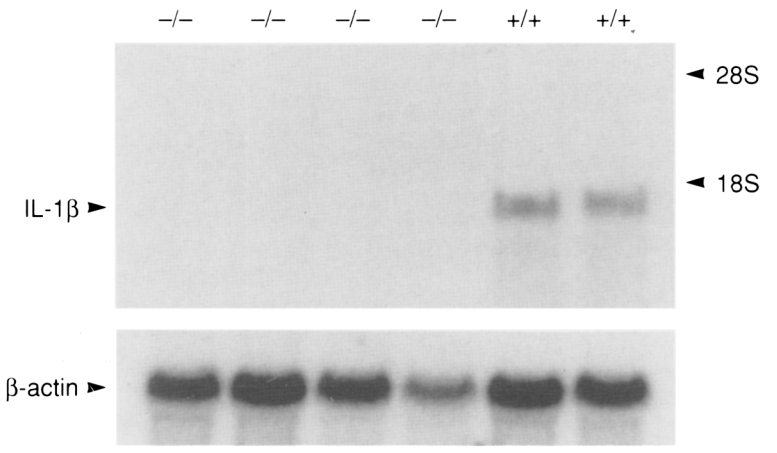

Figure 2. Northern Blot Analysis of IL-1β Expression Following LPS Induction

Liver RNA was isolated from wild-type (+/+) and homozygous (−/−) IL-1β mice 3 hr after LPS injection using RNAzol B method (Biotecx Laboratories, Incorporated) and hybridized with full-length IL-1β cDNA sequence. IL-1β mRNA could not be detected in −/− mice following LPS injection. The lower panel shown in each case is a control hybridization with mouse β-actin cDNA. Northern blot analysis was also performed using unchallanged mice. IL-1β expression could not be detected in control, untreated wild-type, and IL-1β knockout mice (data not shown).

phase proteins tested between the wild-type and the IL-1β-deficient mice (data not shown). This result is similar to that reported for LPS treatment of IL-6 knockout mice (Kopf et al., 1994; Fattori et al., 1994), suggesting that other cytokines, such as IL-1α and TNFα, are able to induce these acute-phase proteins in the absence of IL-1β or IL-6.

IL-1β-Deficient Mice Are Resistant to Turpentine-Induced Weight Loss

Subcutaneous injection of turpentine into mice causes formation of a local sterile abscess and tissue injury, and results in a loss of body weight and synthesis of acute-phase proteins. Both IL-1 and IL-6 are induced by turpentine treatment, whereas TNFα is not (Gershenwald et al., 1990). Experiments using IL-6 knockout mice or neutralizing antibodies against IL-1 receptor reduced the inflammatory response induced by turpentine (Gershenwald et al., 1990; Fattori et al., 1994). In our experiments, the body weight of both the wild-type and IL-1β knockout mice were followed and the change of body weight after turpentine injection was determined (Figure 3). As expected, the control mice lost a significant amount of weight following turpentine treatment. Decreased body weight correlates with decreased food and water intake (data not shown). However, the IL-1β-deficient mice were resistant to turpentine-induced weight loss and anorexia, the body weight and food and water intake being in the same range as untreated mice (Figure 3; data not shown). The weight gain in the first day and weight drop in the third day in the knockout mice apparently result from the extravasation and subsequent resorption of edema fluid at the local turpentine injection site as determined by observation of the swelling, which occurred at the injection site.

The site of turpentine injection was examined microscopically. Both the wild-type and IL-1β-deficient mice exhibited local inflammation characterized by a cuff of inflammatory cells, predominantly neutrophils, surrounding the cyst where turpentine had been injected. There were variable amounts of cellular infiltration, myofiber degeneration, and necrosis in the surrounding skeletal muscles. Although the two groups of mice showed dramatic phenotypic differences in response to turpentine injection, there were no substantial differences in the nature and severity of the lesions between the control and IL-1β knockout mice.

IL-1β Deficiency Results in a Lowered IL-6 Level and a Defective Acute-Phase Response Following Turpentine Treatment

In the wild-type mice, there was a significant increase in plasma IL-6 when measured 8 hr after turpentine injection, but the turpentine-induced IL-6 expression was absent in the IL-1β knockout mice (Figure 4, $p < 0.05$ by student's t test). The IL-1α levels were also analyzed by enzyme-linked immunosorbent assay (ELISA) and these were below the sensitivity of detection in both the wild-type and IL-1β-deficient mice (data not shown).

Inflammatory tissue damage results in the synthesis of hepatic acute-phase proteins. To determine the pattern of acute-phase protein induction following turpentine treat-

Table 1. ELISA Analysis of Cytokine Levels Following LPS Induction

ng/ml ± SEM	IL-1β	IL-1α	TNFα	IL-6
Wild-type	0.60 ± 0.04	0.23 ± 0.03	61.2 ± 6.6	193 ± 13
IL-1β$^{-/-}$	0.00 ± 0.00	0.21 ± 0.05	41.4 ± 12.3	158 ± 41

Both the wild-type (n = 11) and homozygous IL-1β-deficient mice (IL-1β$^{-/-}$) (n = 7) were sensitized by intraperitoneal injection of P. acnes and challenged by intraperitoneal injection of 10 μg LPS 6 days later as described in Experimental Procedures. Plasma and peritoneal fluid were prepared 3 hr after LPS injection and analyzed by ELISA to determine the concentrations of various cytokines. No significant difference was found comparing IL-1α, IL-6, and TNFα levels induced in wild-type and IL-1β knockout mice. The data shown are the result of the analysis of plasma. ELISA analysis of peritoneal fluid produced similar results (data not shown).

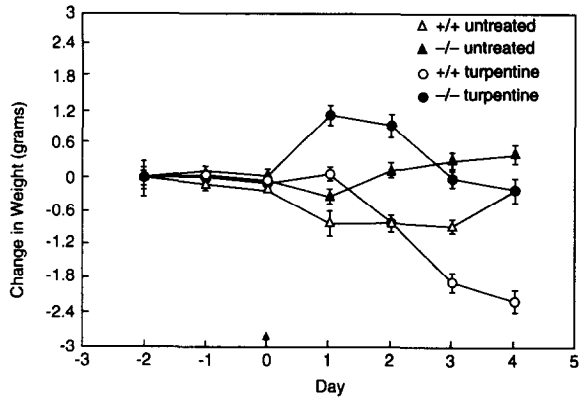

Figure 3. Change in Body Weight Following Turpentine Treatment

The body weight of male mice (n = 10) was measured daily before (days −2, −1), on (day 0), and following (days 1, 2, 3, and 4) turpentine injection. The change in weight relative to day −2 is shown. A group of 10 wild-type and IL-1β mutant mice were not injected and used as untreated controls. Open triangle, wild-type (+/+) untreated; open circle, wild-type (+/+) injected with turpentine; closed triangle, IL-1β knockout mice (−/−) untreated; closed circle, IL-1β knockout mice (−/−) treated with turpentine.

ment, liver RNA was isolated and the level of acute-phase protein expression (SAA, HP, AGP, and α_2m) was quantified by Northern blot analysis. The level of mRNA normalized with β-actin is shown in Figure 5. Overall, there is a 2- to 5-fold reduction in SAA, HP, and α-AGP expression in the knockout mice compared with the wild-type controls. mRNA levels are indicative of the levels of circulating proteins; an earlier report has shown that the serum plasma levels of these acute-phase proteins correlate well with the liver RNA levels (Kopf et al., 1994). We could not detect any α_2m mRNA in the IL-1β-deficient mice, although it was significantly induced in the wild-type animals (data not shown). The overall expression pattern of these acute-phase proteins in the IL-1β null mice is similar to that of the IL-6 knockout mice (Kopf et al., 1994; Fattori et al., 1994), suggesting that the reduction in acute-phase proteins may be secondary to the lack of IL-6 induction in the IL-1β-deficient mice.

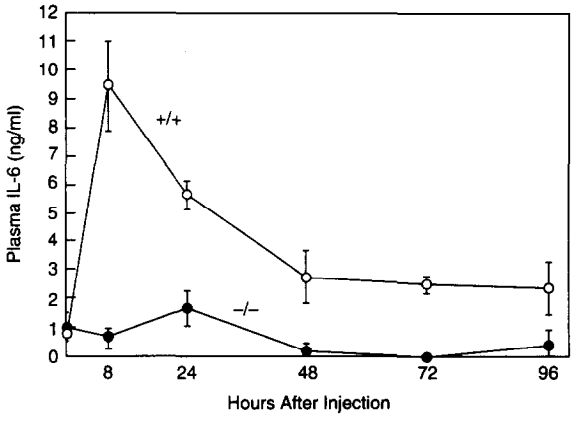

Figure 4. ELISA Analysis of IL-6 Levels Following Turpentine Administration

Plasma (n = 5/timepoint) was prepared from blood taken 0, 8, 24, 48, 72, and 96 hr after turpentine treatment from both the wild-type mice (+/+, open circle) and the IL-1β mutant mice (−/−, closed circle). The IL-6 level was determined by ELISA at different timepoints ($p < 0.05$ comparing wild-type and IL-1β deficient mice at each timepoint, student's t test).

Defective Fever Development in IL-1β Knockout Mice

Both the control and IL-1β-deficient mice were implanted intra-abdominally with battery-operated minitransmitters

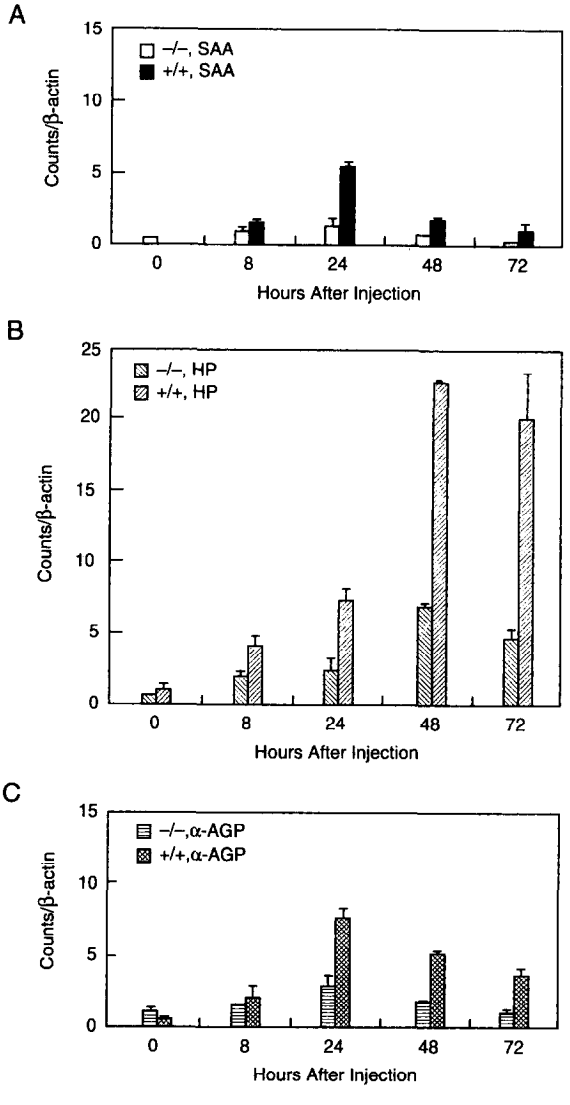

Figure 5. Acute-Phase Protein Expression Following Turpentine Treatment

Liver total RNA was prepared at 0, 8, 24, 48, and 72 hr after turpentine administration (n = 4/timepoint) using standard procedures and hybridized with cDNAs for the following acute-phase proteins: SAA (A), HP (B), α-AGP (C) and α_2m (data not shown). +/+, wild-type controls; −/−, homozygous IL-1β-deficient mice. Probing with β-actin was also performed and used as an internal standard. The values given were the averaged expression of each acute-phase protein normalized with β-actin.

to record body temperature. The mice were injected subcutaneously 2 weeks after surgery with either turpentine or with saline, and the body temperature and locomotor activity were monitored. When control mice were injected with turpentine, they responded with a profound fever, which started within 5 hr of injection and lasted approximately 24 hr (Figure 6A). Their average body temperature reached a peak of 39.4°C ± 0.04°C 20 hr after the injection and stayed elevated up to 35 hr following injection (Figure 6A). Following the fever, the wild-type mice underwent a period of hypothermia, as evidenced by the lack of normal circadian temperature rise during night 2 following the injection (Figure 6B). The increase in body temperature was accompanied by a decrease of locomotor activity (Figure 6C, day 0 to day 1).

In contrast with control mice treated with turpentine, neither body temperature nor locomotor activity were affected at any timepoint following turpentine injection in IL-1β-deficient mice, as evidenced by a complete overlap of both parameters with their saline-injected counterparts (Figures 6A, 6B, and 6C). The 12 hr average of body temperature during night 0 for the IL-1β knockout mice injected with turpentine was 37.1°C ± 0.1°C compared with 39.1°C ± 0.06°C for the turpentine-treated control mice (Scheffe, $p = 0.0001$). The rise in body temperature in the control group and IL-1β-deficient mice at 9 hr and again at 34 hr is due to the normal circadian rise in body temperature. Interestingly, the basal locomotor activity of IL-1β knockout mice was lower than that of the control group (Figure 6C, $p < 0.05$). The reason for this is not clear.

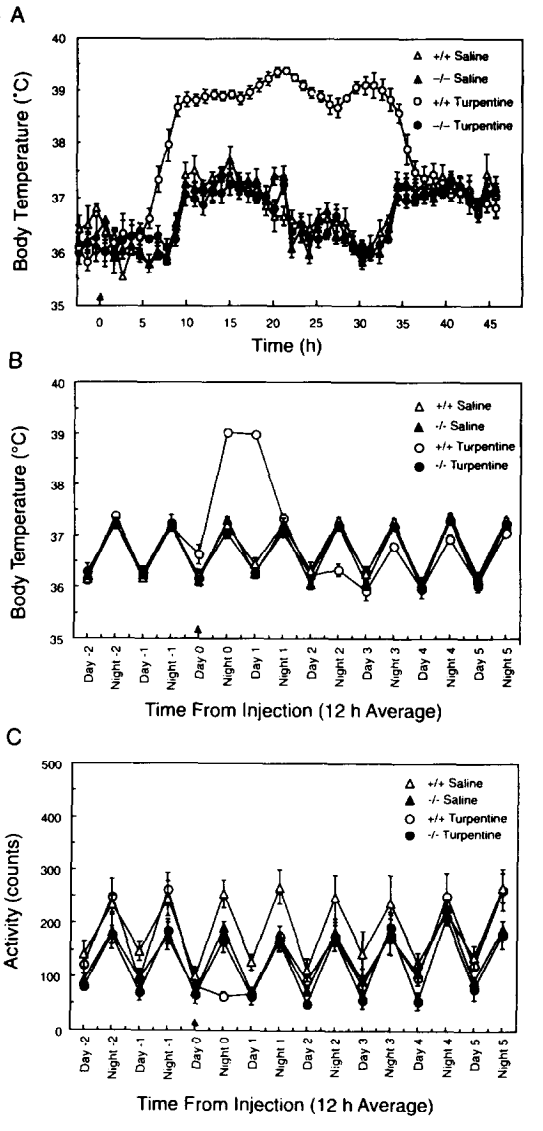

Figure 6. Fever Induction Following Turpentine Injection

129Sv(ev) inbred male mice were implanted with minitransmitters to monitor the body temperature. Mice were injected either with saline (wild-type, open triangle [+/+]; IL-1β knockouts, closed triangle [−/−]; n = 4/group) or with turpentine (wild-type, open circle [+/+]; IL-1β knockouts, closed circle [−/−], n = 5/group). The body temperature and locomotor activity were automatically recorded both before and after injection.

(A) Short-term temperature change following turpentine administration. Note a complete lack of fever induction in the knockout mice following turpentine treatment.

(B) Long-term body temperature measurement before and after turpentine injection.

(C) Locomotor activity of wild-type (+/+) and IL-1β-deficient mice (−/−) before and after turpentine injection.

IL-1β-Deficient Mice Exhibit the Same Response in DTH and CH Reactions

IL-1β has been implicated as a central mediator of DTH reactions. We characterized DTH and CH reactions in IL-1β knockout mice using two substances: the purified protein derivative methylated bovine serum albumin (mBSA) was used for the DTH and the contact allergen oxazalone for the CH. In the DTH reaction, the mice were sensitized by skin injections to mBSA and challenged 7 days later by injection into one hind footpad. The contralateral footpad was injected with saline. The DTH response was measured 24 hr later as the percentage of swelling in the paw injected with mBSA compared with the saline-injected paw (Figure 7A). In addition, a nonsensitized control group was run in the same manner to ascertain the level of nonspecific swelling following mBSA injection. The results show that there is a significant DTH response in both the wild-type and the IL-1β knockout mice (sensitized versus nonsensitized, $p < 0.001$ for both groups), and the mean paw swelling between the control mice and the IL-1β-deficient mice was not statistically different ($p = 0.36$).

The CH reaction was determined by painting oxazalone on the abdominal skin to sensitize the mice. Mice were then challenged by painting oxazalone on one ear 5 days later. The CH response was measured as the difference in weight of biopsied section from the painted ear over the unpainted contralateral ear, determined 24 hr later. An equivalent CH response developed in the IL-1β null mice compared with the wild-type controls (Figure 7B, $p = 0.748$). This result is in direct contrast with previous studies showing that an IL-1β-neutralizing antibody specifically inhibited the CH response in mice (Enk et al., 1993). Taken together, the studies reported here suggest that IL-1β does

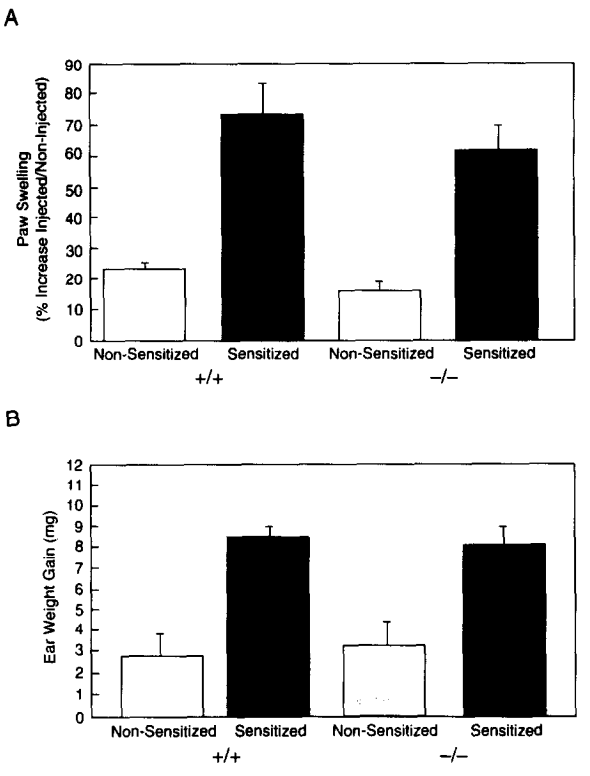

Figure 7. DTH Reactions

(A) DTH to mBSA. Both the wild-type (+/+) and IL-1β-deficient (−/−) mice were either sensitized (closed bar) or nonsensitized (open bar) with mBSA and challenged by subsequent injection of mBSA into the left hindpaw. The DTH response was determined as the degree of swelling in the injected paw in the sensitized group. The value shown in the nonsensitized group represents nonspecific reaction (sensitized versus nonsensitized, $p < 0.001$ for both groups; sensitized, +/+ versus −/−, $p = 0.36$ by student's t test).

(B) DTH to contact allergen oxazolone. Both the control (+/+) and IL-1β knockout (−/−) mice were either sensitized (closed bar) or nonsensitized (open bar) by applying oxazolone on the skin. Mice were challenged 5 days later by painting oxazolone on one ear again. The other ear was not treated and used as a control. The DTH reaction was measured as the weight gain in the painted versus nonpainted ear. The value shown in the nonsensitized group represents nonspecific reaction (sensitized versus nonsensitized, $p < 0.001$ for both groups; sensitized, +/+ versus −/−, $p = 0.748$ by student's t test).

not play a crucial role in either CH or DTH reactions in the mouse.

IL-1β-Deficient Mice Show the Same Susceptibility to Murine Listeriosis

To determine whether IL-1β-deficient mice were impaired in their ability to mount a host defense response to the intracellular pathogen L. monocytogenes, a sublethal dose of 4.1×10^3 organisms was injected intravenously into both the wild-type controls and IL-1β null mice. The mice were sacrificed 4 days after inoculation and the multiplication of L. monocytogenes in both spleen and liver was determined. The results showed that the wild-type and IL-1β knockout mice exhibited equivalent resistance to intravenous challenge with L. monocytogenes. Spleen and liver organism counts were 6.34 ± 0.37 and 4.92 ± 0.30 $\log_{10}$, respectively, in the knockout mice and 6.59 ± 0.22 and 5.13 ± 0.49 $\log_{10}$, respectively, in the wild-type mice ($p = 0.582$ and 0.772 between the wild-type and mutant mice in spleen and liver, respectively; student's t test).

Discussion

IL-1β and the Cytokine Network

IL-1β is regarded as a principal mediator of inflammation. We tested our IL-1β mutant mice against two different inflammatory stimuli used in the experimental models: systemic inflammation caused by LPS injection, and turpentine-induced local tissue injury and inflammation. At various times up to 6 hr following injection of LPS, the levels of IL-1α, IL-6, and TNFα were elevated to the same extent in the IL-1β knockout and wild-type mice, although IL-1β protein was not detected in the circulating blood or peritoneal fluids of the knockout animals (Table 1; data not shown). These results show that IL-1α production is independent of the level of IL-1β in this model. Unexpectedly, the phenotype of LPS-challenged ICE-deficient mice, which are also defective in mature IL-1β production, differs in this regard as it appears that both the plasma levels of IL-1α as well as IL-1β are greatly reduced (Li et al., 1995). Taken together, these data support the idea that interfering with the ability of ICE to process IL-1β affects IL-1α production. Similar to reported results obtained using IL-6-deficient mice, the hepatic acute-phase protein induction was not significantly altered in the IL-1β knockout mice. This result can be explained by cytokine redundancy, i.e., the loss of one cytokine, such as IL-1β or IL-6, can be compensated for by other cytokines, such as IL-1α and TNFα. Since TNFα is the earliest cytokine induced by LPS and is a major factor conferring endotoxin sensitivity to mice (Pfeffer et al., 1993), it is likely that TNFα plays a critical role in LPS-regulated events. Indeed, acute-phase protein elevations in mice injected with LPS were significantly suppressed by treatment of mice with an antibody to TNFα (Harbrecht et al., 1994).

In contrast, TNFα is not induced by turpentine (Gershenwald et al., 1990). Our results show that the turpentine-induced IL-6 elevation is diminished in the IL-1β-deficient mice, suggesting that IL-1β has a primary role in the expression of IL-6 in this model. This may be viewed in the context of the cytokine network, wherein the loss of one cytokine, IL-1β, results in the deficiency of another cytokine, IL-6. However, this does not rule out the possibility that IL-1β acts by more indirect mechanisms to affect IL-6 expression. Likewise, the acute-phase protein expression pattern in the IL-1β knockout mice is similar to that of IL-6-deficient mice (Kopf et al., 1994; Fattori et al., 1994), suggesting that IL-1β indirectly induces acute-phase protein expression through IL-6 up-regulation.

IL-1β and Fever

Body temperature is controlled in the thermoregulatory center of the hypothalamus. Fever results from an elevated hypothalamic set point. Pathogenesis of fever in-

volves the activation of macrophages by various exogenous pyrogens; for example, LPS or turpentine (Cooper and Rothwell, 1991; Kozak et al., 1994), which lead to the synthesis and release of endogenous pyrogens. Although IL-1β is considered to be a major endogenous pyrogen, other cytokines, such as IL-6 and TNFα, have been proposed to play important roles in fever development as well (reviewed by Kluger, 1991). Since the increase in IL-1β is usually accompanied by an increase in the IL-6 level, fever induction following LPS or turpentine treatment in wild-type animals may be caused by IL-1 or by an IL-1-induced IL-6 expression (LeMay et al., 1990; Klir et al., 1993, 1994).

Here, we show that the IL-1β-deficient mice are completely resistant to turpentine-induced fever, demonstrating that IL-1β is critical in fever development. The complete inhibition of fever induction in the IL-1β null mice was surprising, since previous studies using neutralizing antibodies to IL-1β or IL-1ra resulted only in partial suppression of fever (Kluger, 1991). However, since IL-6 is not expressed in the absence of IL-1β following turpentine abscess, the resistance to fever development in the mutant mice could be due to the lack of either or both of these cytokines. In LPS-induced fever development, we show that although IL-6 is expressed at an equivalent level in both the IL-1β-deficient mice and wild-type controls, the temperature rise is suppressed by 50% in the mutant animals (Kozak et al., 1995). This result suggests that although IL-1β itself is essential for fever induction, the development of a full blown fever may require both IL-1β and IL-6. The confirmation of this hypothesis awaits the analysis of fever induction in IL-6-deficient mice.

IL-1β and DTH

DTH response in mice is mediated by antigen-specific T cells and can be initiated by purified protein derivative or by contact allergens (Asherson and Ptak, 1968). Previous studies have shown that the induction of DTH reactions involve the production of several cytokines, such as IL-1α, IL-1β, IL-6, TNFα, and interferon-γ (IFNγ) (Enk and Katz, 1991; Ferreri et al., 1991; Chu et al., 1992). Studies using recombinant IL-1 and neutralizing antibodies have shown that IL-1β may be important in initiating the CH response in skin, whereas blocking of IL-1α and TNFα does not have a dramatic effect (Enk et al., 1993). Our results in models of both DTH to the purified protein derivative mBSA and CH to oxazolone in IL-1β-deficient mice show that IL-1β is not required for either of these responses. Other cytokines, such as IL-1α, IL-6, and TNFα may either compensate for the loss of IL-1β or they may play a primary role in these models. Although the reason for the discrepancy between our results and those described by Enk et al. (1993) is not clear, it raises the possibility that antibodies may elicit a more complex reaction. Our results are similar to those described by Faherty et al. (1992), in which administration of human IL-1ra or a neutralizing antibody to type I IL-1 receptor to mice failed to inhibit a variety of antigen-specific immune responses, including the CH reaction to oxazolone. Our data are in keeping with the large body of evidence suggesting that IL-1 is not required for normal lymphocyte development or responsiveness (Faherty et al., 1992; Arend, 1993). We predict agents that specifically block IL-1β will not be general immunosupressive agents.

IL-1β and Host Defense

Several cytokines have been proposed to be important in promoting immune responses to microbial pathogens. Murine resistance to the intracellular facultative bacterium L. monocytogens is a well-characterized model for this type of host defense and requires both T cell–dependent and independent activation of macrophages. IL-1, as well as IL-6, TNFα, and IFNγ have been shown to be involved in this mechanism. In support of this, mice deficient for IL-6, TNF receptor p55, and IFNγ all showed a higher susceptibility to L. monocytogenes infection (Dalton et al., 1993; Pfeffer et al., 1993; Kopf et al., 1994). Our IL-1β-deficient mice, however, exhibited the same resistance to L. moncytogenes as the wild-type controls, suggesting that IL-1α may be sufficient in murine resistance against Listeria. These data are in keeping with the failure of a neutralizing monoclonal antibody to IL-1β alone to potentiate Listeriosis in mice (Rogers et al., 1992). In contrast, IL-1β seems to be required for host defense against influenza virus infection, since the IL-1β-deficient mice showed a higher mortality rate following intranasal inoculation of influenza virus as compared with wild-type controls (Kozak et al., 1995). This is consistent with the observations that certain viruses produce potent inhibitors of IL-1β processing or soluble receptors, which preferentially bind IL-1β (Alcami and Smith, 1992; Ray et al., 1992; Spriggs et al., 1992). These results suggest that different cytokine networks are customized by the host and utilized for defense against different organisms.

In this report, we explored the role of IL-1β in several inflammation models and we show that IL-1β is a crucial factor in the fever and anorexia development during localized inflammation induced by turpentine. In this model, IL-1β appears to control the expression of IL-6. Either IL-1 alone or the combination of IL-1 and IL-6 contribute to the acute-phase responses following turpentine injection, including loss of body weight, acute-phase protein induction, and, most dramatically, fever development. However, in the systemic inflammation model induced by LPS injection and in DTH response, IL-1β does not seem to be essential. IL-1 has been implicated in a large number of disease models, such as islet destruction (Corbett and McDaniel, 1995), cartilage destruction (Geiger et al., 1993), and allograft rejection (Fanslow et al., 1990). The IL-1β knockout mice reported here should provide a valuable tool to address the function of this cytokine and its role as a target for pharmaceutical intervention in these and other models of inflammation and disease.

Experimental Procedures

Construction of IL-1β Deletion Vector and Generation of IL-1β Null Mice

The mouse IL-1β genomic sequence was isolated by screening a mouse cosmid library derived from 129Sv strain of ES cells using mouse IL-1β cDNA as a probe (Telford et al., 1986). The isolated clone

contained the entire coding region of the IL-1β gene as well as 5′ promoter region of the gene.

The 1.3 kb KpnI–BglII fragment encoding exon 7 of the IL-1β gene was made blunt and cloned into the blunt-ended EcoRI site of pGEM7(tk) vector containing the PGK–*tk* cassette. A 1.8 kb PGK–*neo* fragment was inserted upstream of the 1.3 kb IL-1β sequence. A 4.0 kb BamHI fragment containing 5′ noncoding region of the IL-1β gene was inserted upstream of the *neo* cassette. A 5.6 kb sequence from BamHI site to KpnI site encoding part of exon 1 to intron 6 of the IL-1β gene was deleted. The final targeting vector was linearized by SalI digestion, which cuts downstream of the *tk* sequence prior to electroporation.

Electroporation and blastocyst injection were performed as described (Zheng et al., 1995).

Animal Care and Treatment

Mice were housed in a virus antibody-free facility and the care of animals were conducted as approved by the Institutional Animal Care and Use Committee of Merck and Company, Incorporated. Mice of mixed C57BL/6J and 129Sv(ev) background were used for all studies except fever experiments, in which case inbred 129Sv(ev) strain was used, which were produced by breeding the chimeras with 129Sv(ev) females (Taconic).

For LPS injection, both the wild-type and IL-1β knockout mice about 8 weeks old (n = 7) were sensitized by intraperitoneal injection of 1 mg P. acnes in 1 ml saline and challenged by intraperitoneal injection of 10 μg LPS in 0.5 ml saline 6 days later to induce the expression of IL-1β and other cytokines. Mice were sacrificed 3 hr after challenge by CO_2 asphyxiation. Heparinized blood was obtained by cardiac puncture. The peritoneal cavities were lavaged with 2 ml heparinized saline. Both the cell-free plasma and lavage fluids were prepared by centrifugation and stored at −70°C until cytokine analysis by ELISA. Liver RNA was prepared from the same mice and Northern hybridizations were carried out by standard procedures using the mouse IL-1β cDNA as a probe.

In the turpentine-induced sterile abscess model, male mice at approximately 9 to 10 weeks of age (n = 10/group) were injected subcutaneously with 100 μl of commercial grade steam-distilled wood turpentine (Sunneyside Corp., Wheeling, Illinois) into the left hindlimb. At 0, 8, 24, 48, 72, and 96 hr following turpentine treatment, the body weight was measured, and groups of mice were sacrificed by CO_2 asphyxiation. A group of 10 mice of both the wild-type and knockout animals were maintained as noninjected controls and their body weight was determined at the same timepoints. Heparinized blood was collected by cardiac puncture. Plasma was prepared and used to determine the IL-6 levels. Liver RNA was isolated and used for analysis of acute-phase protein expression.

For temperature measurements and fever induction, the deep body temperatures of the mice were measured with an accuracy of ± 0.1°C using a battery-operated biotelemetry device (model VMFH MiniMitter, Sunriver, Oregon) implanted intra-abdominally using aseptic surgical conditions. The transmitters (dimensions: 13 × 18 mm; weight, 2.5 g) were coated with surgical wax, calibrated, and soaked overnight in Cidex for sterilization before surgery. For implantation, mice were anesthetized with halothane, a small incision was made through the skin and abdominal muscles (~1.5 cm), and then the transmitter, first rinsed with a pyrogen-free isotonic sodium chloride, was implanted into the abdominal cavity. Muscle and skin were sutured separately with absorbable Vicryl sutures and the animals allowed to recover 14 days before experimentation. Experiments were started after a regular rhythm of body temperature and locomotor activity in freely moving mice had been monitored for at least 3 days. Recordings were made at 5 min intervals using a peripheral processor (Dataquest III System) connected to an IBM personal computer. After 2 weeks, mice were divided into four groups, anesthetized with halothane, and injected (subcutaneously) either with steam-distilled turpentine (n = 5) or with saline (n = 4) (100 μl per animal) into the left hindlimb. Body temperature measurements were then continued. The locomotor activity of the mice was measured using the same biotelemetry system described above. In brief, changes in activity were detected by changes in the position of the implanted transmitter over the receiver board. This results in a change in the signal strength that is detected by the receiver and recorded as a "pulse" of activity.

In the DTH reaction to mBSA, 20 IL-1β knockout mice and 20 wild-type controls were used. In each group, 10 mice were sensitized by three subcutaneous injections on the dorsum of each mouse, using a total of 25 μg mBSA in 300 μl saline:CFA (1:1). The remaining 10 mice in each group were not injected and constituted the nonsensitized groups. Each mouse was injected subcutaneously into the left hind footpad with 25 μl of 10 mg/ml mBSA in saline 7 days later. The volumes of both the uninjected right and the injected left hind paw were determined 24 hr later for each mouse using a mercury plethysmograph. The difference between the injected and uninjected paws were calculated and the DTH response was expressed as a percent increase of the injected paw. The nonspecific response is represented by the paw swelling in the nonsensitized groups. Saline-injected paws were found to return to normal volume within 24 hr and, therefore, this control group was not routinely run.

For testing of contact sensitivity to oxazolone, mice were sensitized by shaving the abdominal area of each mouse, applying 100 μl 4% 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone, Sigma, St. Louis, Missouri) in acetone to the skin, and allowing it to dry. Mice were challenged 5 days later by painting both sides of one ear with 50 μl 1% oxazolone in acetone, leaving the contralateral ear as a control. The mice were euthanized 24 hr later by CO_2 asphyxiation. A disc of ear tissue was removed from both ears of each mouse, using a 6 mm biopsy punch, and weighed. The difference between the painted and unpainted ear weights for each mouse was taken as the amount of swelling occurred in response to oxazolone. A nonsensitized control group was run in each experiment to determine the amount of swelling that occurred in response to the ear challenge alone. Results are expressed as the change in weight due to oxazolone challenge ± SEM (n = 10).

For host defense experiment, the wild-type and IL-1β null mice (n = 4/group) were challenged by intravenous infection with 4.1×10^3 L. monocytogenes. The mice were euthanized 4 days later and the organism enumerated as described (Rosen et al., 1989).

Data Analysis

For fever experiment, data were analyzed using Statview SE+Graphics (Abacus Concepts, Berkeley, California). Analysis of variance (ANOVA) with repeated measures was used to determine differences among groups in patterns of temperature and activity changes over time. ANOVA followed by Scheffe pairwise comparisons was used to test for statistical differences among groups at individual timepoints. Analysis of ELISA, DTH, and host defense data were performed by student's t test.

ELISA Assays

ELISAs were developed for murine IL-1α, IL-1β, and IL-6 using antibodies as described in Molineaux et al. (1993). A monoclonal antibody to IL-1β was provided by Dr. D. Chaplin (Washington University, St. Louis, Missouri). A polyclonal antibody to murine TNFα was purchased from Genzyme (Cambridge, Massachusetts). The ELISA sensitivity was 0.05 ng/ml.

Acute-Phase Protein Expressions

Liver RNA from control and IL-1β mutant mice (4 each) was isolated at 0, 8, 24, 48, and 72 hr following turpentine injection by homogenizing the tissue in guanidine isothiocyanate and probed with ^{32}P-labeled cDNAs for mouse SAA (Sipe et al., 1993), rat HP (Baumann et al., 1986), rat α-AGP (Baumann et al., 1986), and rat α_2m (Northemann et al., 1985). The same filters were also hybridized with mouse β-actin for loading control. The intensity of each acute-phase protein was quantified using phosphorimager and normalized to β-actin.

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